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EXAMINER	
TON, THAIAN N	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 04/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/080,713	COLMAN ET AL.	
	Examiner	Art Unit	
	Thai-An N Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 62-90 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 62-90 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 6/17/03 .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' Preliminary Amendment, filed 617/02, has been entered. Claims 62-90 are pending and under current examination.

Priority

An application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification of in an application data sheet (37 CFR 1.78(a)(2) and (a)(5)). The specific reference to any prior nonprovisional application must include the relationship (i.e., continuation, divisional, or continuation-in-part) between the applications except when the reference is to a prior application of a CPA assigned the same application number. In the instant case, this application claims priority to 09/475,674.

Acknowledgment is made of applicant's claim for foreign priority based on an application filed in the United Kingdom on 3/4/99 [9905033.8] and 7/20/99 [9917023.5]. It is noted, however, that applicant has not filed a certified copy of the two U.K. applications as required by 35 U.S.C. 119(b).

Information Disclosure Statement

Applicants' IDS, filed 6/17/03, has been considered.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 62-90 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claimed invention is directed to methods for producing a transgenic animal by modifying the nuclear genome of a somatic cell ad an endogenous locus by a genetic targeting event; transferring the modified nuclear genome of said somatic cell to a recipient cell, thereby producing an animal embryo, and causing the embryo to develop to term, thereby producing a transgenic animal. In further embodiments, the claims are directed to methods for producing transgenic offspring by breeding the resulting transgenic animal.

Note further that the art at the time of filing clearly recognized that some outside event to the donor cell in an NT procedure must occur for successful development of an NT unit. Both reprogramming and nuclear/nucleoli remodeling are events that the art regards as necessary for a cell to be completely totipotent,

i.e., for the cell to become competent to give rise to a live animal. Fulka *et al.* [Reference AT 9 of Applicants' IDS, filed 5/22/02] state that the success when embryonic cells were used as nuclear donor was likely due to the embryonic cells not being completely differentiated at the time of transfer, and thus, amenable to undergo full reprogramming. See p. 848, 1st col, 1st ¶. Fulka further state that complete genomic reprogramming in transplanted nuclei would be accompanied by a sequence of developmental and biochemical changes in the reconstructed embryo that would exactly parallel those detected in normal embryos after fertilization [p. 850, 2nd col., 1st ¶]. Kono *et al.* [Ref. AR 15] state that the breakdown of the nuclear envelope is necessary for reprogramming, as reprogramming probably requires the contact of the chromatin with the ooplasm [p. 76, 2nd col., 2nd ¶]. Wolf [Ref. AR 37] state that the coordination between cell cycles of donor and recipient cell is important to avoid DNA damage and to maintain correct ploidy of the embryo [p. 102, 2nd col.]. They further state, and in support of Kono, that a donor nucleus is reprogrammed by the recipient cytoplasm, where the donor nucleus is reverted to the same morphological and temporal pattern of the zygote [see p. 102, 1st col.]. It is noted that Fulka state that the cloning of adult mammals is very inefficient and highly unpredictable [see p. 849, 1st col., and pp. 850-851, bridging ¶]. Thus, the art at the time of filing, recognized that the cloning of mammals required a process whereby the donor nuclei, by a mechanism that was unclear, was reprogrammed such that the differentiation status of the donor nuclei returned to totipotent. The

art also taught that activation of the embryonic genome occurs at different cell divisions in mammalian embryos, but that reprogramming must be complete by the time of activation. In mice, reprogramming must be complete by the second cell division, in cattle and sheep activation occurs at the fourth cleavage, and reprogramming is believed to occur slowly over the first or second cell cycles. See Fulka, p. 850, 1st ¶, lines 16-25. The specification only discusses serum starvation as a means for reprogramming the donor somatic cell nucleus, and without further guidance as to other means for reprogramming donor cell nuclei, the claims are not enabled for their full breadth.

The claims recite methods of modifying the nuclear genome of a somatic cell at an endogenous locus by a genetic targeting event, and in further embodiments, this even is mediated by homologous recombination. The breadth of the claims encompasses utilizing any somatic cell in the claimed methods. The instant specification contemplates a broad list of suitable somatic cells that would be used in the claimed methods [see p. 5, lines 21-30], and discusses the current limitations in gene targeting in somatic cells; particularly, that homologous recombination in somatic cells is an infrequent event and that it is often necessary to transfect and screen large populations of cells in order to pick a specific clone and that somatic cells have a limited lifespan in culture. See pp. 21-22. The specification teaches that somatic cells that have been shown to support NT are usually of fibroblast origin, although NT has been shown to be successful in cumulus, oviduct and

mammary oviduct and granulosa cells. See p. 27, lines 5-8. The working examples of the specification are directed to gene targeting and subsequent NT of primary ovine fetal fibroblasts [Examples 1-4], gene targeting of primary mammary epithelial cells [Example 5] and the gene targeting of porcine fetal fibroblasts [Example 6], gene targeting in primary bovine fetal fibroblasts [Example 7]. The unpredictability of homologous recombination in somatic cells, as discussed by the instant specification, is supported by the art. Thomson *et al.* [**Reprod. Supp.**, 61:495-508 (2003)] review the state of the art of gene targeting in somatic cells for use in NT methodologies and state that procedures to enhance the lifespan of targeted somatic cells *in vitro* are needed. In particular, Thomson states that premature senescence often occurs, which makes it difficult to confirm a targeting event in somatic cells and that cloning efficiency has been negatively correlated with passage number. See pp. 501. The inefficiency and unpredictability of homologous recombination in somatic cells is supported by Polejaeva and Campbell [**Theriogenology**, 53:117-126 (2000)] who teach that gene targeting in somatic cells is unpredictable because of the lower frequency of homologous recombination than ES cells, and the finite number of cell divisions. They further discuss specific criteria for more efficient somatic cell gene targeting, such as the ability of the cells to have a high single cell-cell cloning efficiency because during drug selection, the cells must be able to expand into clonal cultures. However, they note that human dermal fibroblasts are not able to proliferate under regular culture conditions, and

thus, optimization of culture conditions must be attained for success in somatic cell gene targeting. See p. 120-121. Clearly, the art supports the unpredictability in the gene targeting of any somatic cell for use in NT methodologies, and more specifically, that candidate somatic cells that would be used for gene targeting must be able to survive multiple rounds of cell division, selection and overcome senescence. The specification fails to provide teachings or guidance for utilizing any somatic cell for gene targeting which would be further used in NT methods. Although the state of the art, as well as the specification, supports that particular cell types, such as fetal fibroblasts, could be used in the claimed methods, specific guidance must be provided to enable the breadth of the claims.

The claims encompass methods of NT utilizing any mammalian oocytes with any nuclear donor; however, it is noted that methods of NT require specific methods steps to enable them because the unpredictabilities associated with NT. For example, successful NT requires both ideal recipient oocytes and donor cells, proper activation and embryo culture and implantation into a suitable host animal to produce an animal. The unpredictable state of the art of NT is supported by Campbell [**Cloning & Stem Cells**, 3(4):201-208 (2001)] state that, "Successful development [of the NT unit] is dependent upon numerous factors, including type of recipient cell, source of recipient cell, method of reconstruction, activation, embryo culture, donor cell type, and donor and recipient cell cycle stages." See *Abstract.* The specification teaches that the oocytes used in the working examples are MII

oocytes, however, the breadth of the claims are to oocytes from any stage of development. Campbell teaches that metaphase II [MII] oocytes are considered the cytoplast of choice because the genetic material is arranged upon the meiotic spindle and easily removed [see p. 202, 2nd column, 1st ¶], further, following introduction of the donor somatic cell into an enucleated oocyte, activation must occur to induce further development and the timing of this activation in relation to NT has been implicated in the ability of the NT unit to develop further [see p. 203, 2nd col.]. Fulka *et al.* [**Theriogenology**, 55(6):1373-1380 (2001)] state that the three basic types of cytoplasts 1) enucleated metaphase II oocytes that are used immediately for NT, 2) MII oocytes that are enucleated and aged in culture to be used in NT and 3) oocytes that are first activated and then enucleated in telophase II before use in NT. See p. 1374. Accordingly, the state of the art supports that only oocytes in MII or oocytes in telophase II be used for successful NT. The specification fails to teach or provide guidance for oocytes in other stages of development and in fact, the specification supports that MII oocytes are the cytoplast of choice [see p. 16, lines 11-26].

The claims fail to provide a step of activating the resulting NT unit; thus, without further development of the NT unit, it would not be possible to generate a pluripotent cell. Indeed, the step of activating the NT unit is essential; Dinnyés *et al.* [**Cloning & Stem Cells**, 4(1): 81-90 (2002)] state that, "In NT, the lack of sperm-induced fertilization steps necessitate the application of an artificial activation in

order to trigger further development." See p. 83, 2nd column, last ¶. Dinnyés state that, "NT is a complex procedure, and each step affects the overall efficiency. The unpredictability of the technology due to biological variation of the recipient oocytes and donor cells is difficult to control. Therefore, standardization of steps is important in order to obtain consistent results. Improvements in technical steps may have lasting effects on the development of the fetus." See p. 83, 1st column, 2nd full ¶. The instant claims fail to provide steps of activation or further culture of the NT unit and thus, in view of the state of the art, it would not be predictable that the resulting unit would be able to develop further. The instant specification supports the requirement for an activation step by stating that, "When using MII oocytes as cytoplast recipients the oocytes must be activated by other stimuli." See p. 18, lines 12-25.

The claims fail to provide a step of transferring the resulting NT unit into a surrogate mother. However, the instant specification teaches that the NT unit must then be transferred into a surrogate recipient in order for the animal to be grown to term. See p. 19, lines 2-4. Additionally, the claims fail to be enabling because they do not provide a step of transferring the resulting embryo into a surrogate mother of the same species. Some of the instant claims encompass the implantation of cultured nuclear transfer units into surrogate mothers of different species. The state of the art of implanting embryos into surrogate mothers of different species is unpredictable. For example Fehilly *et al.* (**Nature**, Vol. 307, 16

February 1984) teach that often two unrelated species cannot carry a live hybrid fetus to term due to factors such as interspecific pregnancies, placental abnormalities and maternal immunological reaction against foreign antigens of the conceptus which would be the cause of immediate abortion (see p. 634, 1st column, 2nd paragraph). Fehilly *et al.* summarize experiments for the production of such animals, and show an extremely low percentage of full term young (see Table 1, p. 635). Although Fehilly *et al.* show that is possible to produce embryos that have been implanted into surrogate mothers of a foreign species, it is clearly an unpredictable process.

The breadth of the claimed invention encompasses the cloning humans. These embodiments are not enabling because of the art-recognized inability to clone primates. Vogel [**Science**, 300:226-227 (2003)] state that Rhesus monkey NT-generated embryos seemed normal at their early stages but were unable to develop further when implanted into a surrogate mother. This was because the cells had the wrong number of chromosomes, and that this aneuploidy resulted in the abortion of the fetus. This was found to also be the case with human NT embryos. See p. 225. Simerly *et al.* [**Science**, 300:297 (2003)] state that, "Primate NT appears to be challenged by stricter molecular requirements than in other animals ... With current approaches, NT to produce embryonic stem cells in nonhuman primates may prove difficult – and reproductive cloning unachievable." See p. 297, 3rd column, last sentence. As the state of the art evidences that NT in primates is

unpredictable, and the instant specification fails to provide teachings to show that primate NT using the claimed methods would result in pluripotent mammalian cells, it would have required undue experimentation for one of skill in the art to make and use the claimed invention.

Certain of the instant claims state that the genetic targeting even result in the a gene targeted cell clone:randomly targeted cell clone ratio of equal or greater than 1:100 and that the gene targeting even is carried out at a locus abundantly expressed in the somatic cell. See claims 67-68 and 74 for example. The specification states that homologous recombination frequencies can be increased by selecting a target gene that is abundantly transcribed (p. 7, lines 5-9). The specification only defines abundantly expressed loci as being either the collagen gene locus or the β-lactoglobulin locus. See p. 7, lines 14-17. The general definition of the term "abundantly expressed" is insufficient guidance for the artisan to know particular loci that would meet this definition. See p. 7, lines 9-14. The specification states that the use of an abundantly expressed locus is necessary for the invention, yet there is no guidance as to a locus other than the collagen locus which would provide the claims 1:100 targeted cell clone:randomly targeted cell clone ratio. The specification provides no evidence as to the targeted cell clone: randomly targeted cell clone ration that is obtained when the β-lactoglobulin locus is targeted. Furthermore, the specification fails to provide guidance as to other means of targeting, other than by homologous recombination. The only teaching

provided by the specification is by preparation of a vector for homologous recombination, and without further teachings with regard to characteristics that make a locus abundantly express, what would make a locus capable of the 1:100 target cell clone:randomly targeted cell clone ratio, or by other means of targeting a particular locus, the specification fails to enable the breadth of the instant claims.

The claims are directed to methods of producing a transgenic animal and progeny from transgenic animals. However, the claims fails to provide an appropriate phenotype for the resulting animal and the state of the art of generating transgenic animals is unpredictable with regard to the resulting phenotype. This is because the art of transgenic animals has for many years stated that the unpredictability lies with the site or sites of integration of the transgene into the target genome. Transgenic animals are regarded to have within their cells cellular mechanisms which prevent expression of the transgene, such as DNA methylation or deletion from the genome (Kappell et al (1992) Current Opinion in Biotechnology 3, 549, col. 2, parag. 2). Mullins et al (1993) states that not all animals express a transgene sufficiently to provide a model for a disease as the integration of a transgene into difference species of animal has been reported to given divergent phenotypes (Mullins et al (1993) Hypertension 22, page 631, col. 1, parag. 1, lines 14-17). The elements of the particular construct used to make transgenic animals are held to be critical, and that they must be designed case by case without general rules to obtain good expression of a transgene; e.g., specific

promoters, presence or absence of introns, etc. (Houdebine (1994) J. Biotech. 34, page 281). "The position effect" and unidentified control elements also are recognized to cause aberrant expression (Wall (1996) Theriogenology 45, 61, parag. 2, line 9 to page 62, line 3). Mullins et al.(1996) disclose that "the use of nonmurine species for transgenesis will continue to reflect the suitability of a particular species for the specific questions being addressed, bearing in mind that a given construct may react very differently from one species to another." (Mullins et al (1996) J. Clin. Invest. 98, page S39, Summary). Well-regulated transgenic expression is not frequently achieved because of poor levels or the complete absence of expression or leaky expression in non-target tissues (Cameron (1997) Molec. Biol. 7, page 256, col. 1 -2, bridg. parag.). Factors influencing low expression, or the lack their of, are not affected by copy number and such effects are seen in lines of transgenic mice made with the same construct (Cameron (1997), Molec. Biol. 7, page 256, lines 3-9). These factors, thus, are copy number independent and integration site dependent, emphasizing the role the integration site plays on expression of the transgene (Cameron (1997), Molec. Biol. 7, page 256, lines 10-13). Further, Sigmund (2000) states that the random nature of transgene insertion, resulting founder mice can contain the transgene at a different chromosomal site, and that the position of the transgene effects expression, and thus the observed phenotype (Sigmund (2000) Arteroscler. Throm. Vasc. Biol. 20, page 1426, col. 1, parag. 1, lines 1-7). With regard to the importance of promoter selection, Niemann (1997) states that

transgenic pigs made with different promoters regulating expression of a growth hormone gene give disparate phenotypes - one deleterious to the pig, the other compatible with pig health (Niemann (1997) Transg. Res. 7, page 73, col. 2, parag. 2, line 12 to page 73, col. 1, line 4). While, the intent is not to say that transgenic animals of a particular phenotype can never be made, the intent is to provide art taught reasoning as to why the instant claims are not enabled. Given such species differences in the expression of a transgene, particularly when taken with the lack of guidance in the specification for any transgenic non-human animal whose genome comprises a modification at an endogenous locus by a gene targeting event, it would have required undue experimentation to predict the results achieved in any one host animal comprising and expressing the transgene, the levels of the transgene product, the consequences of that product, and therefore, the resulting phenotype.

Accordingly, for the reasons cited above, it would have required undue experimentation for the skilled artisan to carry out the claimed methods without an a predictable degree of success to implement the invention as claimed.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 62 is incomplete. The claim is directed to a method for producing a transgenic animal by NT; however, the claim fails to provide specific method steps. For example, part (c) of the claim recites that the animal embryo is "caused" to develop to term. It is unclear how an embryo would be caused to develop. Particular steps, such as transfer of the animal embryo to a recipient surrogate mother and the subsequent development of the embryo to term, would be required to produce a transgenic animal. Claims 63-89 are dependent from claim 62.

The term "abundantly" in claims 68 and 74 is a relative term which renders the claims indefinite. The term "abundantly" is not defined by the claims, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is unclear how much expression would constitute abundant expression, as such the claims are indefinite. The terms "abundant" and "abundantly" are relative terms in the absence of some standard of comparison. Thus, it is unclear what constitutes the metes and bounds of these claims.

Claim 90 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: the claim fails to provide specific method steps to produce a transgenic offspring. For example, part (c) of the claim recites "causing" an animal embryo to develop to term. It is unclear how an embryo would be caused to develop. Particular steps, such as the transfer of the

animal embryo to a recipient surrogate mother, and the subsequent development of the embryo to term would be required to produce a transgenic offspring as claimed. Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 62-75, 82-87, 90 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schnieke *et al.* [Ref. AS 26 of Applicants' IDS] in view of Stacey *et al.* [Ref. AT 28] and Stacey *et al.* [Ref. AS 28].

Schnieke teach a method of preparing a somatic cell for NT comprising modifying the genetic material by transfecting ovine primary fetal fibroblasts (somatic cells) with a DNA construct encoding human factor IX operably linked to an ovine β-lactoglobulin promoter, and the subsequent use of the transfected ovine fetal fibroblasts as donors in NT methods. See p. 2130, 3rd col., 2nd ¶, lines 1-7 and p. 2131, 3rd ¶, lines 1-5, and Tables 1-2. The result of the method of Schnieke is the production of sheep transgenic for human factor IX [p. 2132, 2nd col. 2nd ¶]. Schnieke offers motivation in stating that somatic cell donors for NT have advantages over pronuclear microinjection in that fewer animals are needed to produce one transgenic animal, and that no surrogate females are wasted in gestating non-transgenic animals [see p. 2132, 3rd col., 1st ¶]. However, Schnieke do not teach the production of somatic cells from NT by modifying the genetic material of somatic cells by engineering the modification at a precise and predetermined location in the cell's genome. Stacey (1995) both teach the modification of the mouse β-lactoglobulin genomic sequence by a genetic targeting event in mouse ES cells, and a somatic cell (see p. 2835, 2nd col, 6th ¶). Firstly, the β-lactoglobulin gene was replaced by homologous recombination the disrupted β-lactoglobulin sequence.

Then, the disrupted sequence was replaced, also by homologous recombination, with the human β-lactoglobulin gene operably linked to the mouse β-lactoglobulin gene promoter [Stacey (1995), p. 2836, 1st col., 4th ¶]. These procedures inactivate, remove, modify, and replace the mouse β-lactoglobulin gene, while also inserting the human β-lactoglobulin transgene. The insertion of the human β-lactoglobulin gene in the mouse genome cause the upregulation of the expression of human β-lactoglobulin mRNA, which was on average 15-fold higher than mouseβ-lactoglobulin mRNA (see p. 2837, 1st col., 1st ¶, lines 12-14). The β-lactoglobulin locus is described in the specification as being abundantly expressed, and the mouse β-lactoglobulin promoter is an endogenous promoter of a milk gene. As the endogenous β-lactoglobulin promoter directs abundant expression, it would obviously do so in any cell type, such as fibroblast cells, and endothelial cells, as discussed in the specification. Lipofection is one art-known method of introducing DNA into a cell of interest. Stacey (1994) teach plasmids, circular DNA, as being involved in the gene targeting even (see p. 1010, Figure 1). Given the teachings of Schnieke using primary fibroblasts, the use of primary endothelial cells, epithelial cells or muscle cells would have been obvious to the ordinary artisan at the time of filing. Thus, at the time of the instant invention, it would have been obvious for one of ordinary skill in the art to prepare a somatic cell for NT comprising modifying the genetic material of the somatic cell by homologous recombination to inactivate, remove, modify, upregulate or replace a gene, to produce a transgenic NT embryo

which would then be used to produce a transgenic animal, given the combined teachings.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the Examiner be unavailable, inquiries should be directed to Amy Nelson, Acting SPE of Art Unit 1632, at (571) 272-0804. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 872-9306.

TNT

Thaian N. Ton
Patent Examiner
Group 1632

Deborah Crouch

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